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## **--Introduction--**

The research performed under this contract directly supports the U.S. Army Medical Research and Materiel Command (USAMRMC) mission to assess the health effects and hazards of non-ionizing electromagnetic radiation from laser systems. The data obtained will support evaluation of current permissible exposure limits promulgated by TEMED 524 and the ANSI Z-136 laser safety standards. The research addresses three main hypotheses: 1) Damage from 1.55  $\mu\text{m}$  radiation is thermal. 2) Damage from sequences of pulses is cumulative and is correlated by a power law relating the threshold irradiance to the number of pulses in the sequence, and 3) exposures only slightly above the damage threshold for the corneal epithelium will result in damage to the corneal endothelium for these penetrating wavelengths. The hypotheses will be tested by: 1) determining corneal epithelial damage thresholds for single- and multiple-pulse exposures as functions of irradiance, exposure duration, and beam size for wavelengths near 1.55  $\mu\text{m}$ . 2) developing and validating damage models for these wavelengths, and 3) determining thresholds for endothelial damage for single-pulse exposures as functions of irradiance, exposure duration, beam size, and position on the cornea and investigating the healing response for exposures above the epithelial damage threshold.

## **--Body--**

### ***Methodology***

#### ***M1 - Laser System***

An Erbium fiber amplifier driven by a laser diode was used for the exposures (both were supplied by TeraBeam Inc.).\* This laser emits mid-infrared radiation at a wavelength of 1.54  $\mu\text{m}$  and operates in the TEM<sub>00</sub> mode. The amplifier's wavelength was verified by measuring its output with a SPEX Minimate spectrometer equipped with a 300 line/mm grating. The diverging output of the fiber amplifier (NA=0.11) was collected with a 63 mm focal length biconvex glass lens. Corneas were positioned past the focus. Mode quality was verified by direct viewing of the beam on a fluorescent screen and by profiling with a knife-edge at the position where the cornea would be located to verify the Gaussian profile and to determine the 1/e beam diameter.<sup>1,2</sup>

Power was measured with a Scientec Astral AD30 detector and exposure duration was controlled with a Uniblitz shutter. The shutter was calibrated by measuring the passage of a He-Ne laser beam of similar diameter with a photodiode.

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\* Although we purchased an IPG Photonics ELD-10-1550 fiber laser this year, we chose to continue using the laser that had been supplied by TeraBeam for determining thresholds for exposures having durations between 1 and 100 sec to complete the threshold determinations for shorter duration pulses that were called for in the year 1 Statement of Work. The new laser will be used for multiple-pulse exposures in year 2.

## *M2 - Animals*

New Zealand white rabbits of either sex weighing 1.8 – 2.3 kg were used for the experiments. The rabbits were anesthetized with an intramuscular injection of xylazine (12 mg/kg) and ketamine hydrochloride (40 mg/kg). A topical anesthesia (proparacaine hydrochloride 1/2%) also was applied to each eye and a drop of homatropine bromide 5% was instilled to dilate the pupil. A dilated pupil facilitates examining the exposed corneas for minimal lesions. The anesthetized animals were placed in a conventional holder where they were positioned with the aid of a low-power He-Ne laser whose beam was aligned to be coaxial with the 1.54  $\mu\text{m}$  laser beam. The eyes were positioned so that the incident beam was perpendicular to the central cornea. A removable jig attached to the optical bench was used to ensure that the anterior surface of the cornea was located exactly at the position where the beam diameter was determined. A speculum was inserted in the eye about one minute prior to exposure to hold the eye open. In order to create a reproducible tear film, the eye was irrigated with a small amount of physiological saline solution (BSS - Alcon Surgical) that was at room temperature. Irrigation was stopped about 20 sec before exposure and the excess fluid was blotted at the limbus. The corneal surface was assumed to have returned to its normal temperature at the time of exposure. One-half hour after exposure, the rabbits, still under anesthesia, were sacrificed with Beuthanasia-D (100 mg/kg) administered in an ear vein. The eyes were enucleated and examined for damage using a Nikon photo slit-lamp microscope..

## *M3 - Damage Determination*

The criterion we use to determine minimal epithelial damage is the presence of a superficial, barely visible, gray-white spot that develops within 1/2 hour after exposure.<sup>3</sup> Corneas were assessed for damage by examination with a Nikon photo slit-lamp. Near the damage threshold the faint diffuse spot is best observed with a slit width somewhat larger than the damage area.

In these experiments the damage threshold was well defined and there were no overlaps between exposures that produced minimal lesions and those that did not. Therefore statistical procedures such as probit analysis were not used to determine the threshold, as these would have required using more animals than necessary.<sup>4,7</sup> One exposure was made per eye, initially attempting to find broadly bracketing exposures above and below threshold. The bracket was then narrowed until there was only about a 10% difference in irradiance between an exposure that produced a minimal lesion and one that did not. The injury threshold was taken to be at the center of the bracket.

## *M4 - Temperature Calculations*

Temperature calculations are based on a time-dependent Green function solution to the heat equation for the case in which a Gaussian profile laser beam incident on a semi-infinite slab is absorbed according to the Beer-Lambert law.<sup>8-10</sup> The calculations neglect heat transferred from the epithelial surface to the air via convection, radiation, and evaporation. This assumption was justified previously.<sup>11</sup> The calculations also ignore the possibility of convection in the anterior chamber that may be produced by this penetrating radiation, particularly for exposures lasting several seconds. The thermal properties of cornea are assumed to be the same as water.<sup>12, 13</sup> The absorption coefficient,  $\alpha$ , at 1.54  $\mu\text{m}$  was assumed to be 12.3  $\text{cm}^{-1}$ , which is the value for

physiological saline.<sup>14</sup> This value of  $\alpha$  was used because the temperatures were calculated just under the tear layer and also to provide a direct comparison with previous studies that used the appropriate value for saline.<sup>6, 12, 15, 16</sup> The solution for the temperature increase  $\Delta T(r, z, t)$ , where  $r$  is the radial distance from the beam axis,  $z$  is the depth into the cornea, and  $t$  is time, has the form of a definite integral that can be evaluated numerically. The temperature increase  $\Delta T(r, z, t)$  is directly proportional to the incident irradiance. Thus we calculate  $\Delta T(r, z, t)$  for an incident irradiance of 1 Watt/cm<sup>2</sup> and determine the temperature increases for different exposure conditions by multiplying by the appropriate irradiance.

## ***Results and Discussion***

The Year 1 Statement of Work was:

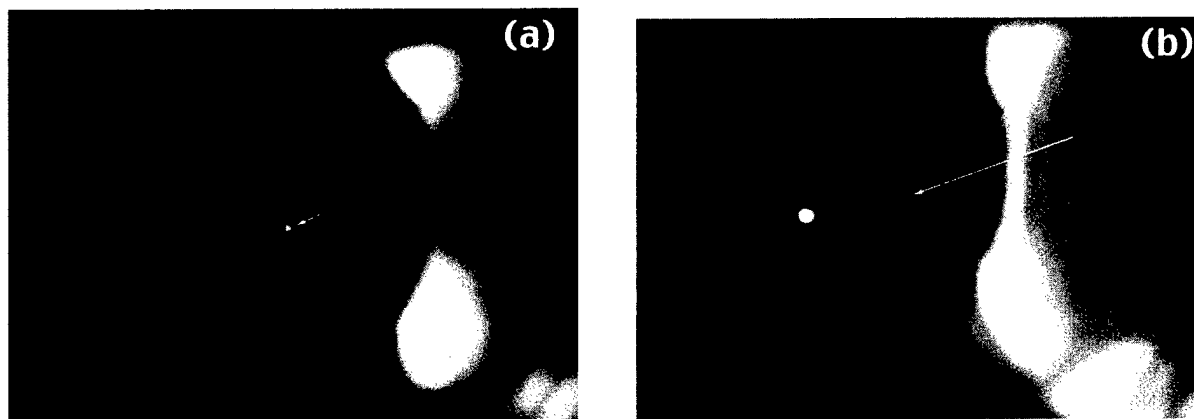
1. The laser will be purchased. Following receipt of the laser we will verify its power output and stability and measure its beam characteristics.
2. We will measure damage thresholds for four exposure durations less than 0.5 seconds (e.g., 0.025, 0.05, 0.1, and 0.25 seconds). The thresholds will be determined for a beam diameter of 1 mm.
3. The development of theoretical damage models will be advanced by examining the effect of including induced convection in the anterior chamber in the thermal model.
4. We will begin determining thresholds for multiple-pulse exposures.

As stated in the proposal we purchased an IPG Photonics model ELD-10-1550 Erbium fiber laser. This laser has a maximum power output of 10 W at a wavelength of 1.55  $\mu\text{m}$ . The laser was prepared for external pulse modulation to facilitate the multiple-pulse experiments. The power output of the laser was verified and its output beam viewed on a fluorescent screen, but the other beam characterizations have been deferred until the beginning of Year 2. We also have deferred work on items 3 and 4 to Year 2.

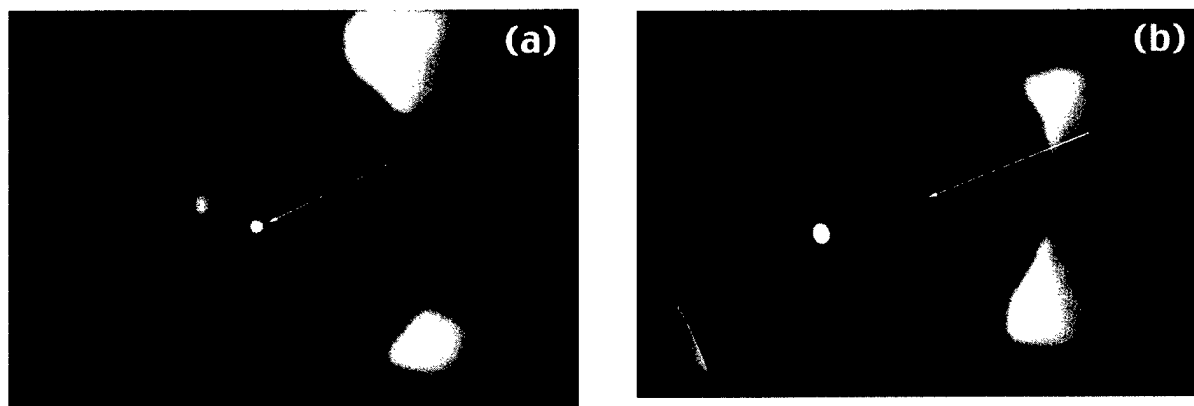
In the revised Statement of Work submitted January 24, 2002 we noted that, subsequent to submitting our proposal, we received funding from a telecommunications company (TeraBeam Corporation) to determine damage thresholds for 1.54  $\mu\text{m}$  laser radiation at four beam diameters for exposure durations  $\geq 1$  sec. It is noteworthy that we received the TeraBeam contract based on Dr. David Sliney's recommendation. Dr. Sliney is Program Manager of the Laser/Optical Radiation program at the U. S. Army Center for Health Promotion and Preventative Medicine. The experiments done with support from TeraBeam Corporation were originally proposed as item 2 of the Year 1 Statement of Work. Therefore in order to avoid duplication, we revised item 2 by proposing to measure injury thresholds for four exposure durations less than 0.5 seconds. Because of laser power limitations these were to be done for a 1 mm diameter beam.

We have completed item 2 of the first year Statement of Work. Thresholds were determined for exposure durations,  $\tau$ , of 0.24sec, 0.10 sec, 0.045 sec and 0.025 sec. The determinations required 30 eyes from 15 rabbits. Figures 1a and 1b show lesions produced by exposures having durations of 0.10 sec. The exposures for the lesions in Figures 1a and 1b were

show lesions produced by exposures having durations of 0.025 sec. The exposures for these lesions were respectively 2.41 and 1.054 times the 0.025 sec damage threshold. As expected the lesions that are only slightly above the damage threshold are very faint and their diameters are much less than those of the greater exposures.



**Figure 1.** Lesions produced by 0.10 second exposures to 1.54  $\mu\text{m}$  radiation from a Er:YAG fiber laser. The exposure parameters were: (a)  $H = 13.9 \text{ J/cm}^2$ ,  $d_{1/e} = 1 \text{ mm}$ ; (b)  $H = 9.79 \text{ J/cm}^2$ ,  $d_{1/e} = 1 \text{ mm}$ . These are respectively 1.47 and 1.038 times the 0.10 sec damage threshold.



**Figure 2.** Lesions produced by 0.025 second exposures to 1.54  $\mu\text{m}$  radiation from a Er:YAG fiber laser. The exposure parameters were: (a)  $H = 11.1 \text{ J/cm}^2$ ,  $d_{1/e} = 1 \text{ mm}$ ; (b)  $H = 4.85 \text{ J/cm}^2$ ,  $d_{1/e} = 1 \text{ mm}$ . These are respectively 2.41 and 1.054 times the 0.025 sec damage threshold.

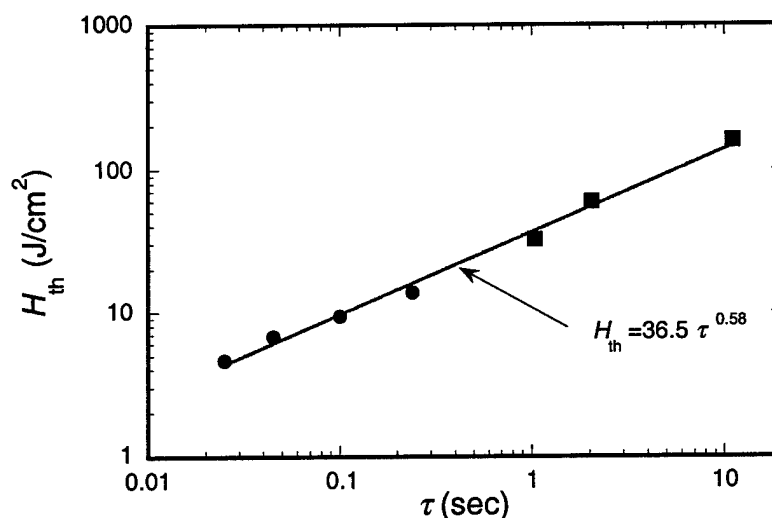
The threshold radiant exposures and irradiances are compiled in Table 1. The radiant exposure threshold values from the table and reference<sup>17</sup> are plotted in Figure 3 as a function of exposure duration where it is shown that the threshold radiant exposure is related to the exposure duration by a power law.

exposure duration where it is shown that the threshold radiant exposure is related to the exposure duration by a power law.

**Table 1 : Damage Thresholds**

$\tau$ (s)	$d_{1/e}$ (cm)	$H_{th}$ (J/cm <sup>2</sup> )	$I_{th}$ (W/cm <sup>2</sup> )	$\Delta T$ (C) <sup>a</sup>
0.24	0.100	13.8	57.4	27.4
0.10	0.100	9.43	94.3	22.4
0.045	0.099	6.75	150	17.5
0.025	0.099	4.60	184	12.4

<sup>a</sup> Calculated on the beam axis, 10  $\mu$ m beneath the anterior tear surface.



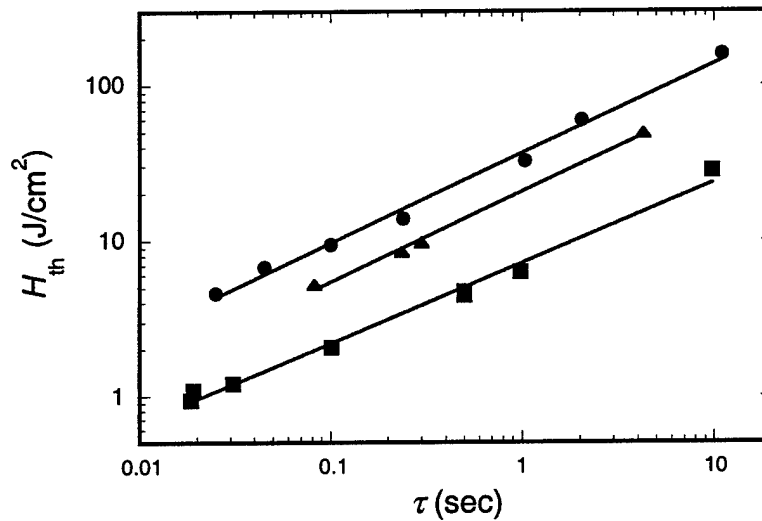
**Figure 3.** The circles are the values of threshold radiant exposures listed in table 1 and the squares are threshold radiant exposures for a 1 mm diameter beam taken from reference 17. The line is a least squares fit to a power law of the form shown in the figure. The R value of the fit was 0.99.

Figure 4 shows that the threshold radiant exposures for 1.54  $\mu$ m radiation are consistent with those for CO<sub>2</sub> laser radiation (10.6  $\mu$ m)<sup>16</sup> and Tm:YAG laser radiation (2.02  $\mu$ m).<sup>11, 18</sup> The absorption coefficients for CO<sub>2</sub>, Tm:YAG and Er:YAG radiation are respectively 950 cm<sup>-1</sup>, 54 .9



$\text{cm}^{-1}$ , and  $12.3 \text{ cm}^{-1}$ <sup>14</sup> and the respective absorption lengths are  $10.5 \mu\text{m}$ ,  $182 \mu\text{m}$  and  $775 \mu\text{m}$ .<sup>†</sup> Thus, as would be expected, wavelengths having smaller absorption lengths where the incident energy is more confined have lower values for threshold radiant exposure.

Table 1 also lists the calculated temperature increases at a point on the beam axis,  $10 \mu\text{m}$  below the anterior tear surface that would result from the threshold exposures. Assuming that the tear film has a thickness of about  $7 \mu\text{m}$ , this point is just inside the anterior-most epithelial cells.<sup>19</sup> As discussed in detail below, these values for the maximum temperature increase at the damage threshold are difficult to understand in light of previous results for Er:YAG thresholds and for  $\text{CO}_2$  and Tm:YAG thresholds.



**Figure 4.** Comparison of threshold radiant exposures for  $\text{CO}_2$  (squares),<sup>16</sup> Tm:YAG (triangles),<sup>6, 18</sup> and Er:YAG (circles)<sup>17</sup> (and Table 1) laser radiation. The  $1/e$  beam diameter was 1 mm for the Er: YAG and Tm: YAG exposures and 2 mm for the  $\text{CO}_2$  exposures. Although the absorption coefficients vary over nearly two orders of magnitude (from  $12.8 \text{ cm}^{-1}$  for Er:YAG to  $959 \text{ cm}^{-1}$  for  $\text{CO}_2$ ) the slopes of the plots are nearly identical.

In the case of the Er:YAG thresholds determined in the study funded by TeraBeam Corporation the predicted temperature increases for the 1.04, 2.05, 11 and 100 s exposures averaged 32.0, 39.4, 36.8 and 32.8 C, respectively.<sup>20</sup> These results suggested that the thresholds could be described by a critical temperature damage model. Similarly, previous studies found that epithelial damage thresholds for exposures to single pulses of  $\text{CO}_2$  and Tm:YAG laser radiation were consistent with either a critical temperature damage model or a modified critical

<sup>†</sup> The absorption length is the distance into the medium at which the irradiance is diminished to  $1/e$  ( $\approx 0.37$ ) of its incident level.

temperature damage model as first described by Egbert and Maher.<sup>6, 13, 16, 18</sup> For single-pulse Tm:YAG exposures between 0.082 and 4.28 sec, the average critical temperature increase calculated at the same position noted above was  $45.8^{\circ} \pm 4.2^{\circ} \text{C}$  (mean  $\pm$  SD).<sup>18</sup> Thus the calculated maximum temperature increases for the single-pulse Tm:YAG exposures were found to be constant to within the experimental uncertainties of  $\pm 10$  percent. Similarly, for CO<sub>2</sub> laser exposures between 0.01 and 1 sec, the maximum temperature increases calculated at the same position were found to be nearly constant with an average value of  $40 \pm 2^{\circ} \text{C}$  (mean  $\pm$  SD). However in the more extended exposure range between 0.001 sec and 10 sec the maximum temperature increases for CO<sub>2</sub> exposures have a weak dependence on exposure duration, varying from 54 to 35  $^{\circ} \text{C}$ .<sup>16</sup> Barger et al (1989) showed that the CO<sub>2</sub> damage thresholds are correlated over the entire range of exposure durations by a modified critical temperature model having the form

$$CPT_{CO_2} = 72\tau^{-0.020} \text{ }^{\circ} \text{C}, \quad (1)$$

in which  $\tau$  is the exposure duration in seconds and  $CPT$  is the critical peak temperature (not temperature increase). This equation assumes that the ambient temperature of the cornea's anterior surface is 35  $^{\circ} \text{C}$ <sup>21</sup> and is similar to the modified critical temperature damage model of Egbert and Maher.<sup>13</sup> Similarly, McCally and Barger<sup>18</sup> showed that if multiple-pulse threshold data for Tm:YAG exposures was analyzed together with the data for single pulses, the Tm:YAG threshold peak temperatures are also fit by a modified critical temperature model given by

$$CPT_{Tm:YAG} = 76D^{-0.054} \text{ }^{\circ} \text{C}, \quad (2)$$

again assuming that the cornea's temperature is 35  $^{\circ} \text{C}$ . Thus epithelial injury thresholds for Er:YAG laser radiation (for exposure durations  $\geq 1$  sec) and CO<sub>2</sub> and Tm:YAG laser radiation can be described by similar critical temperature or modified critical temperature damage models.

The temperature increases listed in Table 1 are not consistent with these models. First, they are significantly lower and second, unlike the modified critical temperature damage models described in equations 2 and 3, they decrease as the exposure duration becomes shorter. The reason for this behavior for the Er:YAG exposures shorter than 1 sec is not understood. The results are particularly perplexing in view of the data shown in Figures 3 and 4. These figures show that the threshold radiant exposures (and therefore the threshold irradiances) for the shorter Er:YAG exposures are consistent with those of the longer Er:YAG exposures and also with the CO<sub>2</sub> and Tm:YAG threshold exposures. Clearly our objective stated in item 3 of the Statement of Work has gained importance, however convection in the anterior chamber would not provide an explanation of these perplexing results.

In other work two manuscripts were prepared with partial support from this contract. One entitled "Corneal Epithelial Injury Thresholds for Multiple-pulse Exposures to Tm:YAG Laser Radiation at 2.02  $\mu\text{m}$ " has been accepted by Health Physics and the other, entitled "Corneal Epithelial Injury Thresholds for Exposures to 1.54  $\mu\text{m}$  Radiation," will appear in the Proceedings of the SPIE, Volume 4953 Laser and Non-coherent Light Ocular Effects: Epidemiology, Prevention, and Treatment.

### **--Key Research Accomplishments--**

- Damage thresholds were determined for single-pulse exposures to 1.54  $\mu\text{m}$  radiation for exposure durations ranging from 0.025 to 0.24 sec.
- Threshold radiant exposures have a power law dependence on exposure duration for durations from 0.025 to 11 sec.
- The results call into question the uniform applicability of critical temperature or modified critical temperature damage models.

### **--Reportable Outcomes--**

#### *Manuscripts*

1. R. L. McCally and C. B. Barger, "Corneal Epithelial Injury Thresholds for Multiple-pulse Exposures to Tm:YAG Laser Radiation at 2.02  $\mu\text{m}$ ," Health Phys. (in press)
2. R. L. McCally, J. Bonney-Ray, and C. B. Barger, "Corneal Epithelial Injury Thresholds for Exposures to 1.54  $\mu\text{m}$  Radiation" to be published in the Proceedings of the SPIE, Volume 4953 Laser and Non-coherent Light Ocular Effects: Epidemiology, Prevention, and Treatment.

#### *Presentations*

1. R. L. McCally and C. B. Barger, "Corneal Epithelial Damage Thresholds for Multiple-pulse Exposures to 2.02  $\mu\text{m}$  Radiation from a Tm:YAG Laser," Laser Bioeffects Meeting, Paris, France, June 13,-14, 2002 (invited).
2. R. L. McCally and J. Bonney-Ray, "Corneal Epithelial Injury Thresholds for Exposures to 1.54  $\mu\text{m}$  Radiation – Dependence on Beam Diameter," Laser Bioeffects Meeting, Paris, France, June 13,-14, 2002 (invited).
3. R. L. McCally, J. Bonney-Ray, and C. B. Barger, "Corneal Epithelial Injury Thresholds for Exposures to 1.54  $\mu\text{m}$  Radiation," Photonics West, BIOS 2003, San Jose, CA, Jan. 25-31, 2003 (invited).
4. R. L. McCally, J. Bonney-Ray, and C. B. Barger, "Corneal Epithelial Injury Thresholds for Exposures to 1.54  $\mu\text{m}$  Radiation," International laser Safety Conference 2003, Jacksonville, FL, March 10-13, 2003 (invited).

## --Conclusions--

Corneal epithelial damage thresholds were determined for single-pulse exposures to 1.54  $\mu\text{m}$  radiation from an Er:YAG fiber laser. The exposure durations were 0.24 sec, 0.10 sec, 0.045 sec and 0.025 sec and the 1/e diameter of the laser beam was 1 mm. When combined with threshold data for exposures having durations between 1.04 and 11 sec, the threshold radiant exposures are described by a power law of the form  $H_{\text{th}} = 36.5 \tau^{0.58} \text{ J/cm}^2$  where  $\tau$  is the exposure duration. Similar relationships (i.e., power laws having the same dependence on  $\tau$ ) characterize threshold damage from  $\text{CO}_2$  and Tm:YAG lasers for exposure durations in the same range. This is particularly interesting in view of the fact that the absorption coefficients span a range of nearly two orders of magnitude. However, unlike damage from Er:YAG exposures having durations  $\geq 1$  sec and damage from  $\text{CO}_2$  and Tm:YAG lasers, the damage thresholds for the shorter Er:YAG exposures are not correlated by either a critical temperature or a modified critical temperature damage model. The temperature rises resulting from the threshold exposures with durations  $\leq 0.24$  sec are not approximately constant and are substantially less than the critical temperatures for the other lasers and the longer duration Er:YAG exposures. Moreover, unlike the modified critical temperature damage models that describe  $\text{CO}_2$  and Tm:YAG laser damage over a wide range of durations, the temperatures associated with damage for the exposures  $\leq 0.24$  sec decrease rather than increase as the exposure duration becomes shorter. The apparent breakdown of the critical temperature damage models for these exposures is perplexing, and needs to be understood.

The single-pulse thresholds for shorter duration pulses reported on here will provide a basis for interpreting the multiple-pulse data that we will obtain in year 2.

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